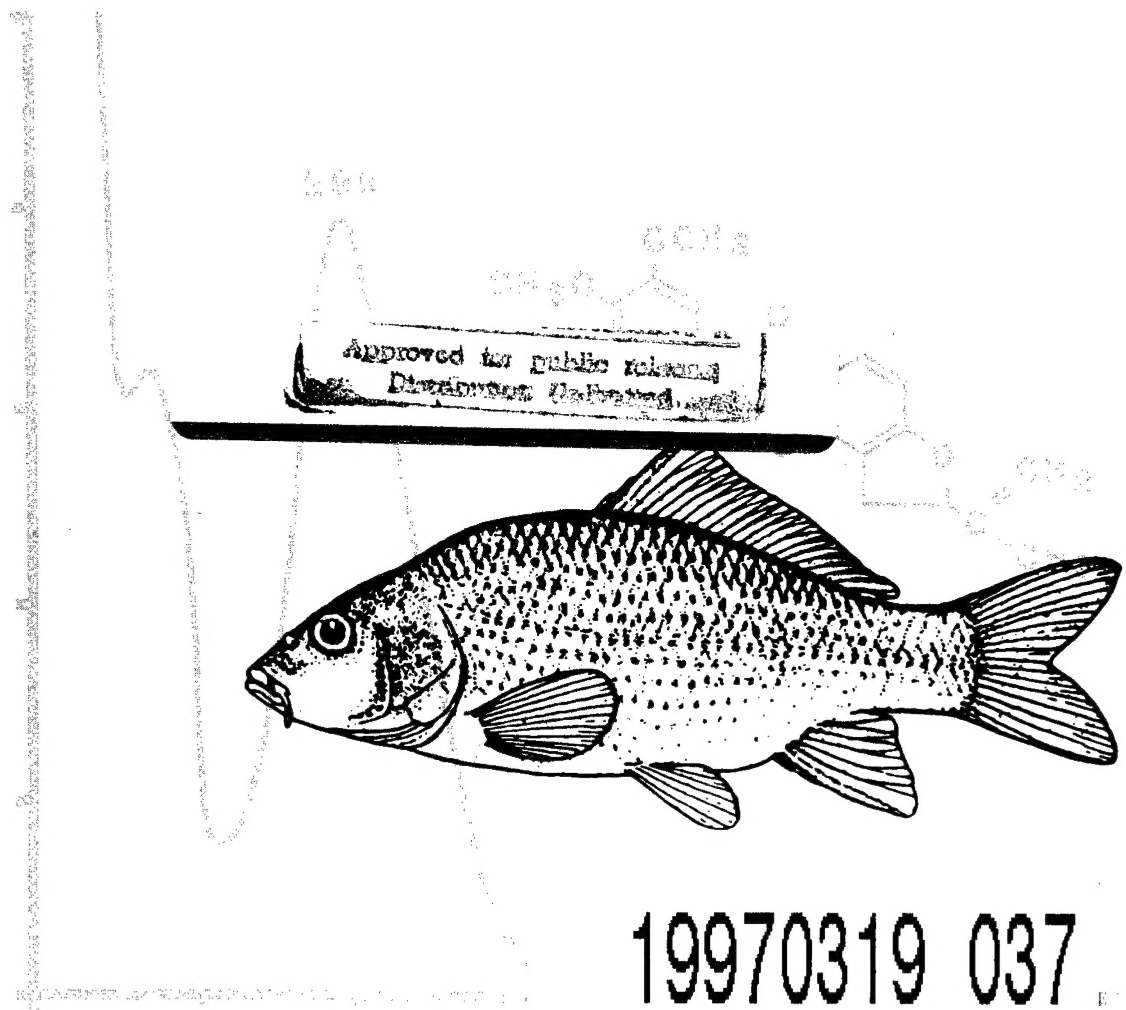
Sensitivity of Juvenile Striped Bass to Chemicals Used in Aquaculture



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by

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Abstract. Efforts to restore anadromous striped bass (*Morone saxatilis*) populations by the U.S. Fish and Wildlife Service and other agencies over the past 20 years have concentrated on hatchery culture to supplement dwindling natural reproduction. Adult fish captured for artificial spawning are stressed by handling and crowding in rearing ponds and are often exposed to therapeutants, anesthetics, disinfectants, and herbicides used in fish culture. We determined the toxicity of 17 fishery chemicals (chloramine-T, erythromycin, formalin, Hyamine 3500, Roccal, malachite green, sulfamerazine, benzocaine, etomidate, Finquel [MS-222], metomidate, quinaldine sulfate, chlorine, potassium permanganate, Aquazine, copper sulfate, and Rodeo) to striped bass fry (average weight = 1 g) in reconstituted water (total hardness 40 mg/L) at 12° C. The 96-h LC50's (concentration calculated to produce 50% mortality in a population) ranged from 0.129 mg/L for malachite green to 340 mg/L for erythromycin. We also determined the effects of selected levels of water temperature, hardness, and pH on the toxicity of chloramine-T, formalin, malachite green, and Roccal. There were no differences in toxicity for any of the chemicals at any water quality variable tested except for chloramine-T, which was about 25 times more toxic in soft, acid water than in soft, alkaline water. Our data show that the striped bass is as sensitive to fishery chemicals as rainbow trout (*Oncorhynchus mykiss*), but is generally less resistant than bluegill (*Lepomis macrochirus*) and channel catfish (*Ictalurus punctatus*).

Key words: Anesthetics, disinfectants, herbicides, *Morone saxatilis*, striped bass, therapeutants, toxicity.

The striped bass (*Morone saxatilis*) also known as rockfish, rock, or linesides, has been an important resource since the colonization of America (Setzler et al. 1980). The range of the striped bass originally extended along the east coast of North America from the St. Lawrence River south to Florida and west to Louisiana (Raney et al. 1952). Artificial culture of striped bass began in the late 1800's when yearlings were transported to the West Coast and stocked in San Francisco Bay (Parker 1984). A hatchery was constructed on the Roanoke River at Weldon, N.C., in 1881, and striped bass were stocked along the Atlantic coast to supplement natural populations. However, interest in striped bass culture waned from the early 1900's to the 1950's, when stocking in reservoirs in the southeastern United States produced a popular inland striped bass fishery. This develop-

ment, along with a greatly depleted coastal striped bass fishery, caused fishery agencies to reevaluate the striped bass program (Stevens 1979).

Since the 1970's, the U.S. Fish and Wildlife Service and other natural resource agencies have annually produced from 8 to 10 million striped bass for the restoration of anadromous populations. Striped bass culture requires extensive handling of fish, which results in stress and injury and makes them susceptible to disease and parasites; therefore, a variety of chemicals are used in their culture.

Although striped bass culture is not new, information on the toxicity of fishery chemicals to striped bass is limited. Wellborn (1969) and Hughes (1971) determined the toxicity of several compounds used in striped bass culture. They concluded that striped bass are more sensitive to chemicals than are most freshwater fishes. Our study was conducted to determine the sensitivity of striped bass to 17 chemicals that are commonly used in culture or that have been proposed for such use. The chemicals tested included therapeutants, anesthetics, disinfecting agents, and herbicides.

Materials and Methods

We used static toxicity test procedures described by the Committee on Methods for Toxicity Tests with Aquatic Organisms (1975) and ASTM Committee E-35 on Pesticides (1980). We exposed 10 juvenile (1-g) striped bass to each test concentration of chemical in glass jars containing 15 L of oxygen-saturated test water. Reconstituted test waters were prepared according to standardized procedures to produce the desired water quality. Temperatures were regulated by immersing the test jars in a constant-temperature water bath; tests were conducted at 12, 17, and 22° C.

Striped bass for the study were obtained from the Welaka (Florida) and Genoa (Wisconsin) national fish hatcheries (NFH) and were cultured at the La Crosse (Wisconsin) National Fisheries Research Center. The fish were treated twice weekly with a 2% sodium chloride bath for 1 h and were acclimated to the desired water chemistries and temperatures for 24 h before each exposure. Observations on mortality were made at 1, 3, and 6 h during the first day of exposure and once daily thereafter for 4 days.

Chemicals and manufacturers or suppliers were as follows: chloramine-T, Badger Pharmacal, Inc.; erythromycin, CEVA Laboratories; formalin, Argent Chemical Company; Hyamine 3500, Rohm and Haas Company; Roccal, Hilton-Davis Chemical Company; sulfamerazine, Argent Chemical Company; benzocaine, Aldrich Chemical; etomidate, Tavolet Chemical Company; Finquel (MS-222), Ayerst Laboratories, Inc.; metomidate, Janssen Pharmaceutical Company; quinaldine sulfate, McLaughlin Gormley King Company; chlorine (HTH), Olin Corporation; malachite green, potassium permanganate, and copper sulfate, Fisher Scientific Company; Aquazine, Ciba-Geigy Corporation; and Rodeo, Monsanto Agricultural Products Company.

All 17 chemicals were tested in soft water at 12° C on fish from Welaka NFH. Six chemicals were tested on fish cultured at the Genoa NFH, which represented a different year class and culture location, to determine if either affected their sensitivity to chemicals. Four chemicals—chloramine-T, formalin, malachite green, and Roccal—were tested on fish from the Genoa NFH in waters of different temperature, hardness, and pH to determine if changes in water characteristics affect sensitivity to chemicals in juvenile striped bass. The methods of Litchfield and Wilcoxon (1949) were used to compute the LC50's (concentration producing 50% mortality) and 95% confidence intervals.

Sensitivity to Fishery Chemicals

Of the 17 chemicals tested, 16 were toxic (Table 1). Sulfamerazine, a bactericide for treatment of kidney disease, was the only chemical that did not cause mortality. Concentrations of ≥100 mg/L formed a precipitate that settled to the bottom of the test vessel. We concluded that saturation was achieved before a lethal level could be reached.

Malachite green, a therapeutant, was the most toxic chemical tested. The 96-h LC50 was 0.192 mg/L in soft water at 12° C. Bills et al. (1977b) reported that the 96-h toxicity of malachite green to nine fish species ranged from 0.0305 mg/L

Table 1. *Toxicity of 17 fishery chemicals to striped bass in soft water at 12° C.*

Chemical	Use	LC50 (mg/L) and 95% confidence interval				
		1 h	3 h	6 h	24 h	96 h
Chloramine-T	Therapeutant	1,230 1,021–1,482	300 236–382	91.0 72.8–114	14.1 11.4–17.5	6.65 5.68–7.78
Erythromycin	Therapeutant	>665	>665	>665	>665	349 266–457
Formalin[a]	Therapeutant	3,320 2,361–4,668	1,410 1,138–1,748	940 809–1,092	211 171–260	75.0 60.3–93.2
Hyamine 3500	Therapeutant	14.2 11.5–17.6	6.90 6.25–7.61	4.70 3.97–5.57	2.82 2.27–3.50	1.41 1.04–1.91
Roccal	Therapeutant	16.4 13.8–19.5	8.05 6.85–9.47	5.00 4.12–6.06	2.77 2.22–3.46	1.90 1.44–2.51
Malachite green	Therapeutant	>10.0	8.79 7.88–9.01	1.06 0.84–1.34	0.28 0.20–0.39	0.19 0.13–0.27
Sulfamerazine	Therapeutant	>100	>100	>100	>100	>100
Benzocaine	Anesthetic	32.9 27.6–39.2	28.1 22.7–34.8	28.1 22.7–34.8	28.1 22.7–34.8	28.1 22.7–34.8
Etomidate	Anesthetic	0.90 0.79–1.02	0.69 0.64–0.76	0.48 0.43–0.55	0.28 0.23–0.35	0.28 0.23–0.35
Finquel (MS-222)	Anesthetic	89.9 82.8–97.6	69.5 63.6–76.0	69.5 63.6–76.0	49.0 43.2–55.5	28.2 22.7–34.9
Metomidate	Anesthetic	2.80 2.25–3.48	2.80 2.25–3.48	2.80 2.25–3.48	2.45 1.89–3.17	2.00 1.64–2.44
Quinaldine sulfate	Anesthetic				26.8 23.7–30.2	22.4 20.0–25.1
Chlorine (HTH)	Disinfecting agent	1.41 1.13–1.75	0.98 0.80–1.20	0.61 0.53–0.69	0.35 0.28–0.43	0.35 0.28–0.43
Potassium permanganate	Oxidizing agent	>60	28.2 22.7–34.9	9.20 7.41–11.4	3.52 2.84–4.36	1.58 1.19–2.10
Aquazine	Herbicide	>1,000	>1,000	>1,000	>1,000	822
Copper sulfate	Algicide	>10.0	>10.0	4.09 3.18–5.26	0.75 0.64–0.88	0.23 0.19–0.29
Rodeo	Herbicide	131 103–166	61.5 51.9–72.8	50.0 41.1–60.8	35.4 27.6–45.4	23.5 18.4–30.0

[a]Toxic unit μL/L.

for bluegill (*Lepomis macrochirus*) to 0.383 mg/L for coho salmon (*Oncorhynchus tshawytscha*). A range of LC50's from 0.168 to 0.288 mg/L was recorded for rainbow trout (*O. mykiss*) under varying conditions of water temperature, hardness, and pH. Our data indicate that striped bass are similar to rainbow trout in their sensitivity to malachite green.

Copper sulfate, an algicide, and chlorine, a disinfecting agent, were the next most toxic chemicals to striped bass. The 96-h LC50 was 0.234 mg/L for copper sulfate and 0.351 mg/L for chlorine. Johnson and Finley (1980) reported that 96-h LC50's for copper sulfate ranged from 0.135 mg/L for rainbow trout to 3.51 mg/L for green sunfish (*L. cyanellus*). We tested striped bass in soft water (total hardness 40–44 mg/L as $CaCO_3$). Because the toxicity of copper is affected by variations in water chemistry, especially hardness, we expect that the copper would be less toxic to striped bass in harder water, as has been shown for other species. Marking and Bills (1977) tested the toxicity of chlorine to 12 fish species in standardized tests that were similar to those we used for striped bass. They reported 96-h LC50's that ranged from 0.156 mg/L for channel catfish (*Ictalurus punctatus*) to 1.41 mg/L for black bullhead (*Ameiurus melas*). However, the 96-h LC50's for most of the 12 species tested were between 0.156 and 0.558 mg/L, and are similar to the values we recorded for striped bass.

Etomidate and metomidate, anesthetics, were next in the order of toxicity. The 96-h LC50's were 0.282 and 2.00 mg/L, respectively. Both anesthetics were 10 to 100 times more toxic than the other anesthetics tested (benzocaine, quinaldine, and Finquel).

The 96-h LC50's for Hyamine 3500 and Roccal, quaternary ammonium compounds used in the treatment of bacterial gill disease, were 1.41 and 1.90 mg/L, respectively. Willford (1966) reported 48-h LC50's for Roccal of 1 to 3 mg/L for six species of fish. Our data indicate that the response of striped bass to these compounds is similar to that of most other species.

Potassium permanganate, a disinfecting and oxidizing agent, was toxic to striped bass at a concentration of 1.58 mg/L (96-h LC50). This agrees with the 96-h LC50's of 1–2.5 mg/L reported by Wellborn (1969) and Hughes (1971) and is similar to the 96-h LC50's reported for rainbow trout, which range from 0.879 to 1.73 mg/L for varying conditions of water temperature, hardness, and pH (Marking and Bills 1975).

The 96-h LC50 for chloramine-T, a therapeutant used to treat bacterial gill disease, was 6.65 mg/L for striped bass. The 96-h LC50's reported for rainbow trout and fathead minnow (*Pimephales promelas*) range from 2.80 to 7.80 mg/L. Our data indicate that striped bass are about twice as resistant to chloramine-T as rainbow trout (Bills et al. 1988).

The anesthetics benzocaine, quinaldine sulfate, and Finquel (MS-222) produced LC50's between 20 and 30 mg/L. These values are similar to those reported by Marking (1967) for MS-222 and by Marking and Dawson (1973) for quinaldine sulfate for several fish species. Our data indicate that concentrations of anesthetics used for other fish should also be safe for use on striped bass.

Rodeo, a nonselective, broad-spectrum contact herbicide, is toxic to striped bass at concentrations of about 25 mg/L. The principal active ingredient in Rodeo is glyphosate (*N*-[phosphonomethyl] glycine). The manufacturer lists 96-h LC50's for Rodeo that range from 86 to 120 mg/L for trout, bluegill, and common carp (*Cyprinus carpio*). Our data indicate that striped bass are significantly more sensitive to Rodeo than are the species listed by the manufacturer.

The 96-h LC50 for formalin, a parasiticide and fungicide, was 75.0 mg/L. This value for striped bass is significantly less than those reported by Bills et al. (1977a) for salmonids and centrarchids, but is similar to the value for channel catfish.

Erythromycin, a therapeutant used for treatment of bacterial kidney disease, was relatively nontoxic and produced a 96-h LC50 of 349 mg/L with striped bass. Erythromycin is administered as a feed additive at 9 to 10 g/100 kg of fish per day. Under these conditions, toxic levels would not be reached even if the feed was not consumed and most of the erythromycin went into solution.

There were no significant differences in the effects of chemicals on striped bass of two different year classes cultured at Welaka and Genoa NFHs. Both groups were exposed to chloramine-T, formalin, Roccal, malachite green, benzocaine, and MS-222 (Table 2).

Table 2. *Toxicity of six fishery chemicals to striped bass cultured at two national fish hatcheries in soft water at 12° C.*

Chemical	Rearing facility	LC50 (mg/L) and 95% confidence interval			
		3 h	6 h	24 h	96 h
Chloramine-T	Welaka NFH	300 236–382	91.0 72.8–114	14.1 11.4–17.5	6.65 5.68–7.78
	Genoa NFH	112 91.4–137	44.0 38.0–51.0	14.4 11.7–17.7	6.30 5.77–6.88
Formalin[a]	Welaka NFH	1,140 1,138–1,748	940 904–1,092	211 171–260	75.0 60.3–93.2
	Genoa NFH	>1,000	760 681–948	120 97.2–148	56.0 45.2–69.3
Roccal	Welaka NFH	8.05 6.84–9.47	5.00 4.12–6.06	2.77 2.22–3.46	1.90 1.44–2.51
	Genoa NFH	6.00 5.15–6.98	2.80 2.40–3.26	1.40 1.20–1.63	1.40 1.20–1.63
Malachite green	Welaka NFH	8.79 7.88–9.01	1.06 0.837–1.34	0.278 0.197–0.392	0.192 0.134–0.275
	Genoa NFH	5.65 4.58–6.98	2.60 2.09–3.23	0.550 0.489–0.619	0.245 0.199–0.302
Benzocaine	Welaka NFH	28.1 22.7–34.8	28.1 22.7–34.8	28.1 22.7–34.8	28.1 22.7–34.8
	Genoa NFH	28.5 25.6–30.6	27.1 25.2–29.2	26.5 24.4–28.8	20.0 17.4–23.0
Finquel (MS-222)	Welaka NFH	69.5 63.6–76.0	69.5 63.6–76.0	49.0 43.2–55.5	28.2 22.7–34.9
	Genoa NFH	59.0 55.7–62.5	49.0 44.8–53.6	49.0 44.8–53.6	49.0 44.1–53.2

[a]Toxic unit μL/L.

Effects of Water Quality on the Toxicity of Selected Chemicals

The toxicity of many chemicals can be affected by differences in water quality. We tested four compounds that are used routinely in fish culture (chloramine-T, formalin, malachite green, and Roccal; Tables 3–6) to determine their effects on striped bass at different levels of water quality.

Our data indicate that striped bass respond to the chemicals and variations in water quality in the same way as other fish. Bills et al. (1988) reported that the toxicity of chloramine-T to rainbow trout was unaffected by water hardness, increased slightly in warmer waters, and increased significantly in acid waters. At 96 h, striped bass were more sensitive in 12° C water than in 22° C water (Table 3). Variations in water hardness did not affect the toxicity of chloramine-T to striped bass, but as water pH decreased, the toxicity increased nearly 20-fold.

The toxicities of formalin and malachite green were not affected by variation in water hardness or pH. However, both chemicals were more toxic to striped bass in warm than in cold waters; the 96-h LC50's at 22° C increased from 56.0 µL/L at 12° C to 30.0 µL/L for formalin (Table 4) and from 0.382 mg/L at 12° C to 0.190 mg/L for malachite green (Table 5). The toxicity of Roccal to striped bass was unaffected at any of the water quality levels we tested (Table 6).

Discussion

Striped bass require special care during spawning and rearing because they are subject to more handling and cultural stress than most other fishes (Stickney 1986). For example, mature striped bass are anesthetized, injected with hormones, and either tank-spawned or manually stripped. Fish that are manually stripped are anesthetized, catheterized, and handled several times before they are spawned because egg samples must be removed periodically to ensure that they do not overripen (Piper et al. 1982). When ovulation occurs in striped bass, the eggs detach from the ovaries and must be removed before 30 min elapse, or the eggs will die from anoxia.

Newly hatched larvae of striped bass are placed in ponds before they are capable of continuous swimming, which makes them vulnerable to predation by aquatic insects. Potassium permanganate is routinely used for pond sterilization before striped bass stocking (Braschler 1975). Potassium permanganate is also used to reduce parasites in ponds before harvest because handling operations during harvest greatly reduce resistance of striped bass to disease (Stickney 1986). The recommended application rate for potassium permanganate is 2 mg/L for use as an oxidizer and detoxifier (Schnick et al. 1989). Our data indicate juvenile striped bass can tolerate up to nearly 10 mg/L for 6 h. The chemical reaction of potassium permanganate with oxidizable matter present in ponds is almost instantaneous. The recommended application is not likely to have any effect on striped bass present in a pond.

Most fungal and parasitic diseases that affect the culture of many warmwater fishes also affect striped bass. Columnaris (*Flexibacter columnaris*), a serious bacterial disease (Hawke 1976), is treated with a variety of chemicals, including diquat, Terramycin, Furanace, copper sulfate, potassium permanganate, and certain sulfonamides. Of this group, copper sulfate, potassium permanganate, and a sulfonamide (sulfamerazine) were tested. When applied at recommended rates, only copper sulfate may become toxic if used in soft water. The application rate for elemental copper is 0.2–1 mg/L (Schnick et al. 1989). Our data indicate a 24-h LC50 of 0.75 mg/L for copper sulfate, which is equivalent to about 0.3 mg/L of elemental copper.

Striped bass are highly sensitive to culture stresses; our data indicate that they are also highly sensitive to fishery chemicals. Striped bass may appear to be similar to rainbow trout in their sensitivity to fishery chemicals. However, differences in toxicity in water of differing pH, hardness, and temperature strongly indicate that individual culture centers should conduct sensitivity tests on small batches of fish, in water from their own facilities, before chemical applications are made to entire raceways or holding ponds.

Table 3. *Toxicity of chloramine-T to striped bass at selected levels of water temperature, hardness, and pH.*

Temperature (° C)	Hardness[a]	pH	LC50 (mg/L) and 95% confidence interval 1 h	3 h	6 h	24 h	96 h
12	Soft (40–48)	7.5	>80.0	112 91.4–137	44.0 38.0–51.0	14.4 11.7–17.7	6.30 5.77–6.88
17	Soft (40–48)	7.5	>80.0	>80.0	49.0 44.8–53.5	14.5 11.8–17.8	9.70 8.70–10.8
22	Soft (40–48)	7.5	81.0 70.2–93.4	48.0 42.6–54.1	28.3 24.3–32.9	9.20 8.23–10.3	8.20 7.18–9.36
12	Very soft (10–13)	8.2	>80.0	>80.0	>80.0	40.0 30.7–52.2	20.0 16.0–25.0
12	Soft (40–48)	8.2	>80.0	>80.0	>80.0	73.0 68.5–77.8	27.8 22.7–34.1
12	Hard (160–180)	8.2	>80.0	>80.0	>80.0	64.0 58.4–70.2	27.6 22.6–33.8
12	Very hard (280–320)	8.2	>80.0	>80.0	>80.0	57.0 53.3–61.0	27.5 22.5–33.6
12	Soft (40–48)	6.5	43.0 36.9–50.1	28.5 23.1–35.2	14.1 12.1–16.4	4.90 4.48–5.35	2.80 2.42–3.24
12	Soft (40–48)	8.5	>80.0	>80.0	>80.0	>80.0	31.5 27.2–36.4
12	Soft (40–48)	9.5	>80.0	>80.0	>80.0	>80.0	52.0 47.9–56.5

[a]Total hardness expressed as mg/L $CaCO_3$.

Table 4. *Toxicity of formalin to striped bass at selected levels of water temperature, hardness, and pH.*

Temperature (° C)	Hardness[a]	pH	LC50 (mg/L) and 95% confidence interval 1 h	3 h	6 h	24 h	96 h
12	Soft (40–48)	7.5	>1,000	>1,000	760 681–948	120 97.2–148	56.0 45.2–69.3
17	Soft (40–48)	7.5	>1,000	>1,000	455 385–538	86.0 74.7–99.0	48.0 36.7–62.8
22	Soft (40–48)	7.5	>1,000	750 642–877	210 160–276	82.0 72.3–93.0	30.0 24.9–36.1
12	Very soft (10–13)	8.2	>1,000	>1,000	600 549–656	87.0 74.9–101	43.0 36.2–51.1
12	Soft (40–48)	8.2	>1,000	>1,000	620 567–678	113 92.8–138	52.0 44.1–61.3
12	Hard (160–180)	8.2	>1,000	>1,000	640 582–704	113 94.2–136	64.0 55.5–73.7
12	Very hard (280–320)	8.2	>1,000	>1,000	660 547–796	110 88.2–137	53.0 45.9–61.2
12	Soft (40–48)	6.5	>1,000	>1,000	510 446–583	140 113–174	59.0 47.8–72.8
12	Soft (40–48)	8.5	>1,000	>1,000	520 450–601	108 95.0–123	66.0 59.3–73.4
12	Soft (40–48)	9.5	>1,000	1,080 990–1180	410 353–477	96.0 60.1–115	48.0 39.1–59.0

[a]Total hardness expressed as mg/L $CaCO_3$.

Table 5. *Toxicity of malachite green to striped bass at selected levels of water temperature, hardness, and pH.*

Temperature (° C)	Hardness[a]	pH	LC50 (mg/L) and 95% confidence interval: 1 h	3 h	6 h	24 h	96 h
12	Soft (40–48)	7.5	>16	5.65 4.58–6.98	2.60 2.09–3.23	0.550 0.489–0.619	0.245 0.199–0.302
17	Soft (40–48)	7.5	11.2 9.10–13.8	2.80 2.41–3.26	1.50 1.23–1.82	0.420 0.362–0.487	0.210 0.180–0.244
22	Soft (40–48)	7.5	5.60 4.81–6.52	1.33 1.12–1.58	0.830 0.693–0.994	0.329 0.281–0.385	0.138 0.093–0.205
12	Very soft (10–13)	8.2	12.8 9.58–17.1	4.30 3.73–4.96	1.80 1.53–2.12	0.425 0.365–0.495	0.190 0.164–0.220
12	Soft (40–48)	8.2	>16	4.90 4.07–5.89	1.88 1.62–2.18	0.425 0.365–0.495	0.213 0.184–0.246
12	Hard (160–180)	8.2	>16	4.75 4.01–5.63	1.89 1.62–2.20	0.422 0.365–0.488	0.185 0.143–0.240
12	Very hard (280–320)	8.2	>16	4.29 3.68–5.00	1.58 1.31–1.90	0.348 0.292–0.413	0.210 0.180–0.244
12	Soft (40–48)	6.5	>16	4.75 3.99–5.65	1.35 1.13–1.61	1.13 1.00–1.26	0.382 0.317–0.460
12	Soft (40–48)	8.5	>16	5.05 4.20–6.07	1.69 1.43–2.00	0.350 0.296–0.414	0.215 0.185–0.250
12	Soft (40–48)	9.5	>16	5.65 4.85–6.57	1.78 1.51–2.10	0.255 0.214–0.303	0.190 0.146–0.248

[a]Total hardness expressed as mg/L $CaCO_3$.

Table 6. *Toxicity of Roccal to striped bass at selected levels of water temperature, hardness, and pH.*

Temperature (° C)	Hardness[a]	pH	LC50 (mg/L) and 95% confidence interval 1 h	3 h	6 h	24 h	96 h
12	Soft (40–48)	7.5	8.00 7.03–9.10	6.00 5.15–6.98	2.80 2.40–3.26	1.40 1.20–1.63	1.40 1.20–1.63
17	Soft (40–48)	7.5	11.4 9.27–14.0	4.70 3.91–5.65	2.80 2.38–3.30	1.40 1.20–1.63	1.40 1.20–1.63
22	Soft (40–48)	7.5	9.40 8.55–10.3	2.80 2.41–3.26	2.60 2.14–3.16	1.40 1.20–1.63	1.30 1.08–1.57
12	Very soft (10–13)	8.2	2.50 2.08–3.00	2.40 2.03–2.84	2.23 1.89–2.63	1.40 1.20–1.63	0.880 0.826–0.937
12	Soft (40–48)	8.2	3.70 2.86–4.81	2.90 2.30–3.65	2.40 2.01–2.87	1.23 1.09–1.39	0.810 0.737–0.890
12	Hard (160–180)	8.2	6.80 6.30–7.34	5.40 4.98–5.85	2.80 2.40–3.26	1.40 1.21–1.62	0.780 0.715–0.851
12	Very hard (280–320)	8.2	8.50 7.60–9.51	6.00 5.55–6.48	3.10 2.75–3.50	1.40 1.20–1.63	0.790 0.722–0.865
12	Soft (40–48)	6.5	6.00 4.70–7.65	4.75 4.22–5.35	3.60 3.18–4.08	1.40 1.20–1.64	1.40 1.20–1.64
12	Soft (40–48)	8.5	11.0 9.17–13.2	5.50 5.08–5.95	2.80 2.42–3.24	1.40 1.20–1.63	1.40 1.20–1.63
12	Soft (40–48)	9.5	11.3 9.15–14.0	5.20 4.76–5.68	2.80 2.28–3.44	1.40 1.20–1.63	1.20 1.06–1.36

[a]Total hardness expressed as mg/L $CaCO_3$.

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